

## HABITUATION OF THE WITHDRAWAL RESPONSE IN NEREID POLYCHAETES

### 1. THE HABITUATION PROCESS IN *NEREIS DIVERSICOLOR*

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The withdrawal response of many polychaetes is easily observable and is elicited by a variety of different stimuli so that it is admirably suited to studies of the habituation process. A rapid withdrawal response is mediated through giant axons in the ventral nerve cord. Five of them have been described in *Nereis virens* by Bullock (1945) and in *N. diversicolor* by Smith (1957) and separate functions have been ascribed to them in *N. virens* by Bullock (1948): the single median fiber normally conducts impulses from the afferent neurones in the anterior region, the paired paramedials conduct from afferents in the posterior region and the paired lateral fibers are excited by stronger stimulation at any point. Each fiber transmits in both directions from the point of excitation.

In studies of habituation of the withdrawal response, sabellids and serpulids have received most attention (*e.g.*, Bohn, 1902; Hargitt, 1906; 1909; Yerkes, 1906; Hess, 1914; Rullier, 1948; Nicol, 1950; Krasne, 1965), but habituation has also been studied in the nereid, *N. pelagica*, by Clark (1960ab). Withdrawal is elicited by several different stimuli in these worms but, in nearly all cases, the response habituates to repeated stimulation although the rate varies according to the nature of the stimulus. Despite the attention habituation of the withdrawal response has received in these worms, little is known about the habituation process itself. Several authors have treated the withdrawal response as an "all or nothing" reflex, and although this may be so in some polychaetes, there is evidence that in *Mercierella enigmatica* (Rullier, 1948) and *Branchiomma vesiculosum* (Krasne, 1965) it is not identical at each stimulus presentation, and that habituation may involve the gradual loss of a response rather than the sudden loss of an "all or nothing" response. From preliminary observations of habituation in *Nereis diversicolor* it was clear that habituation was a complex process in this polychaete and, consequently, it was decided to record successive responses to stimuli by photography so that changes which might occur during habituation, such as in extent of response, rate of contraction and stimulus-response latency could be measured.

Observations on the mud flats populated by *N. diversicolor* showed that habituation is a normal feature of the worm's behavior. Initially individuals feeding on the surface mud are particularly sensitive to sudden stimulation, and great care is needed if an observer is to get sufficiently close to the worms to observe their feeding habits, without eliciting the withdrawal response. Extended worms re-

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spond to mechanical disturbances caused by objects dropped onto the mud, tactile stimuli and shadows. Controlled field experiments are not possible, but habituation can be demonstrated to repeated mechanical disturbances and shadows. Initially the worms almost invariably respond to these stimuli by withdrawing into their burrows, but they re-emerge, usually after several minutes delay, and eventually come to ignore the stimulus.

In the laboratory individuals react to stimuli that are effective in the field and habituate to repeated presentations of them. Observations supported Clark's (1960a) view that withdrawal responses to sudden increases in illumination are different from those to sudden decreases in illumination, moving shadows and mechanical stimuli; they are slower and have longer stimulus-response latencies. Consequently the habituation process has been investigated in *N. diversicolor* by photographing successive responses to sudden decreases in illumination, because it is believed that responses to this stimulus are characteristic of those to a number of other stimuli, and to sudden increases in illumination, which elicit different responses.

#### METHOD

Sixteen specimens of *N. diversicolor* were subjected to consecutive sudden decreases in light intensity, from 30 ft-c to 5 ft-c, at 30 second intervals. In each trial the light was off for two seconds. Responses were photographed using a Paillard-Bolex H. 16 Reflex camera at 32 frames per second. Developed film was examined frame by frame in a film editor; the stimulus-response time latency, time taken to complete contraction, amount of contraction, length of the worm at the onset of each trial and irrigating behavior were recorded for each individual.

General background illumination was provided by reflected light from a 60 watt, white, tungsten-filament bulb in a bench lamp, directed at one of the walls of the dark room in which the experiment was performed. More intense light was provided by a similar bench lamp arranged above the worms; this lamp was switched off at each trial so that there was a sudden decrease in illumination. Light intensities were measured by an S. E. I. exposure photometer using a standard white magnesium carbonate block.

Worms were kept in 70% sea water in a constant temperature room at 16° C for ten days before the experiment. They were provided with 10 cm lengths of glass tubing; only worms inhabiting tubes were selected for experimentation. Within these tubes nereids carry out apparently normal tubiculous behavior (see *e.g.*, Wells and Dales, 1951). In order to facilitate measurement of contractions the tubes containing *Nereis* were placed on millimeter graph paper in an enamelled dish during the experiment. The dish contained 70% sea water and was partially immersed in a constant temperature bath so that the temperature could be maintained at 16° C. Worms were light adapted under the experimental conditions for one hour prior to the first stimulus.

Sixteen specimens of *N. diversicolor* were also subjected to forty sudden increases in illumination from 5 ft-c to 30 ft-c. Apart from the nature of the stimulus the experimental procedure was identical to that described above.

TABLE I

*Stimulus-response latencies and rates of contraction to sudden changes in illumination*

Stimulus:	Sudden decrease in light intensity			Sudden increase in light intensity		
	Nos. observations	Mean rate contraction (mm/sec)	Mean latency (sec)	Nos. observations	Mean rate contraction (mm/sec)	Mean latency (sec)
1	11	17.3	0.42	7	6.5	0.71
1-10	29	12.5	0.43	51	4.7	0.73
11-20	11	9.5	0.45	38	4.2	0.61
21-30	13	8.9	0.42	24	4.2	0.63
31-40	7	10.5	0.38	32	4.5	0.69

## RESULTS

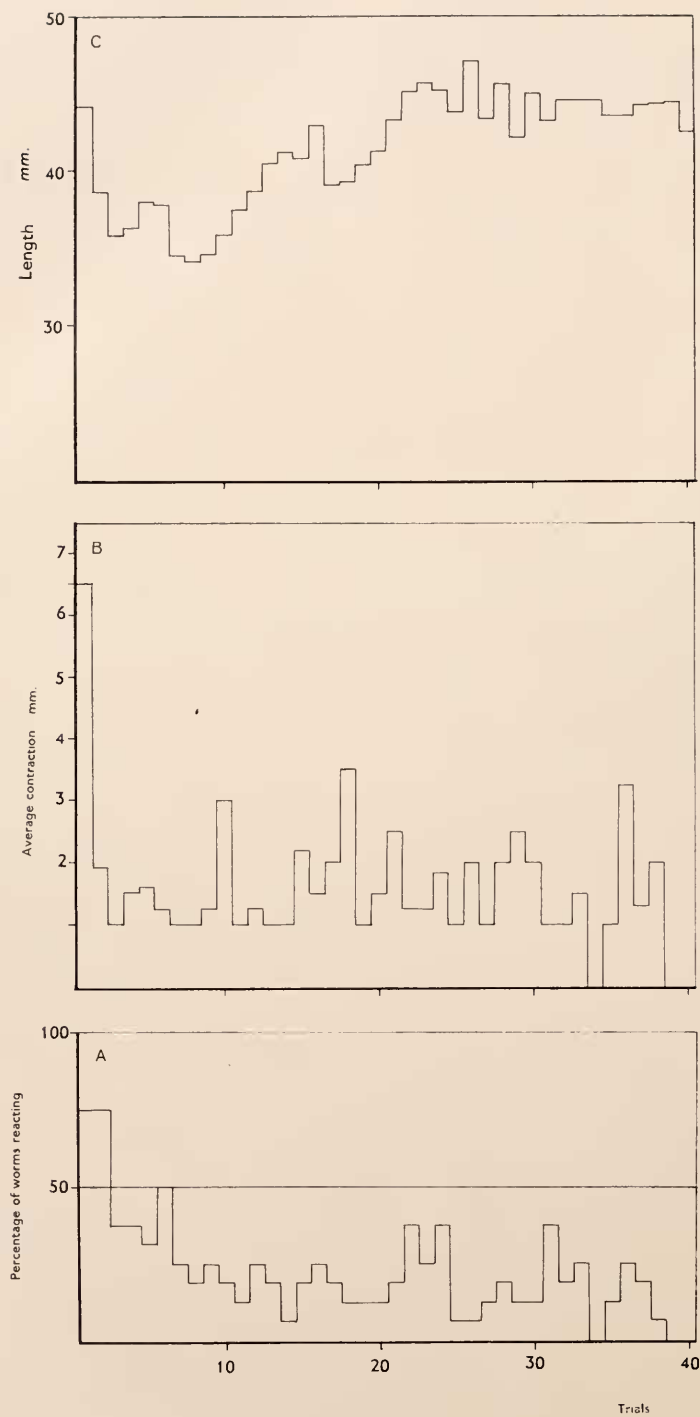
It is clear from the analysis of the photographs that habituation to changes in illumination is a complex process. Habituation to sudden decreases in light intensity can be considered from several aspects:

1. The number of worms responding diminishes with successive trials. Initially 75% of individuals responded to sudden decreases in light intensity, but the number reacting gradually decreased until at the completion of the experiment no worms were responding (Fig. 1A), although it is unlikely that habituation was complete even at this stage.

2. The contraction to the first stimulus was much more extensive than to other stimuli, but subsequently there was no decline in the average extent of contraction of worms (Fig. 1B). In fact, the mean contraction from trials 2-14 (1.44 mm) was actually less than that during the rest of the experiment (1.74 mm). However, any tendency for the extent of contraction to decrease during the first part of the experiment may have been obscured because contractions were limited by incomplete recovery during the intertrial period. Worms which contracted did not extend to their original lengths, and initially there was a cumulative contraction to stimuli (Fig. 1C). Responses were limited during this period, because at each trial the worms were already in a state of at least partial contraction. Subsequently recovery was more rapid, and worms extended to their original lengths during the intertrial period so that maximal contractions were possible.

3. The stimulus-response latencies of contractions of 2 mm or more in length were approximately constant during the experiment and, after the first response, which was the most rapid, the rate of contraction did not apparently diminish (Table I). Responses of less than 2 mm have not been measured because of the inaccuracies of assessing their start and completion.

4. Stimulation not only elicited the withdrawal response in *N. diversicolor* but also inhibited irrigating movements. The responses were partially independent, because although they both involve the longitudinal muscles of the body wall so that a complete contraction necessarily inhibits irrigating activity, incomplete contractions frequently did not inhibit irrigations, and conversely sometimes irrigating movements stopped when there was no contraction. Habituation of the two responses was also independent, and the inhibition of irrigating movements



habituated extremely rapidly. The first three stimulations inhibited irrigation in most of the worms but thereafter inhibition was infrequent (Fig. 2A).

Responses to sudden increases in illumination differed from those to sudden decreases in several ways:

1. Responses to sudden increases were slower and had longer stimulus-response latencies than those to sudden decreases (Table I); the means for both rates of contraction and stimulus-response latencies, calculated from the mean values for each individual worm ( $n = 16$ ), were significantly different in both cases ( $P < 0.01$ ; "t" test).

2. 69.7% of responses were posterior end withdrawals whereas nearly all contractions to sudden decreases in illumination were withdrawals of the anterior end (95.8%).

3. Sudden increases in illumination inhibited irrigation in only 1.3% of trials whereas sudden decreases inhibited it in 16.1% of trials. These differences can probably be related to the fact that irrigating movements are normally confined to the anterior half of the animal, which is usually involved in responses to sudden decreases but not sudden increases in illumination.

In many ways the habituation process to sudden increases in illumination is similar to that already described for sudden decreases, but there are several characteristic differences:

1. The initial sudden decline in numbers responding, which occurred to sudden decreases in illumination, did not occur to this stimulus (Fig. 2B; *cf.* Fig. 1A). Fewer worms responded to the first two sudden increases than sudden decreases, but there were subsequently more responses to the former stimulus, and habituation to it was clearly incomplete at the end of the experiment.

2. For the first six sudden increases in illumination, responses diminished in extent in an irregular manner which contrasts with the sudden loss of the initial extensive contraction to sudden decreases in illumination (Fig. 2C; *cf.* Fig. 1B). Thereafter there was no apparent decline in the extent of contractions to either stimulus. Initially contractions to sudden increases in illumination were cumulative, and recovery was not completed in the intertrial period (Fig. 3A).

3. Apart from the first trial, contractions to sudden increases in illumination were generally more extensive than those to sudden decreases (*cf.* Figs. 1B and 2C).

In other respects the habituation process to sudden increases in illumination was similar to that to sudden decreases.

1. The stimulus-response latencies of responses to sudden increases were longer than those to sudden decreases throughout the experiment (Table I) and, apart from trial 11-20, remained approximately constant.

2. Like contractions to sudden decreases, the first sudden increase in illumination elicited the fastest contraction, although thereafter there was no apparent decline in the rate of contracting (Table I).

Individuals often habituate to either sudden increases or sudden decreases in light intensity in an irregular manner, and sometimes it is not clear from an

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FIGURE 1. A, Percentage number of worms responding to a series of sudden decreases in illumination; B, Mean contractions of worms responding to a series of sudden decreases in illumination; C, Mean lengths of worms, subjected to a series of sudden decreases in illumination, measured at the onset of each trial.

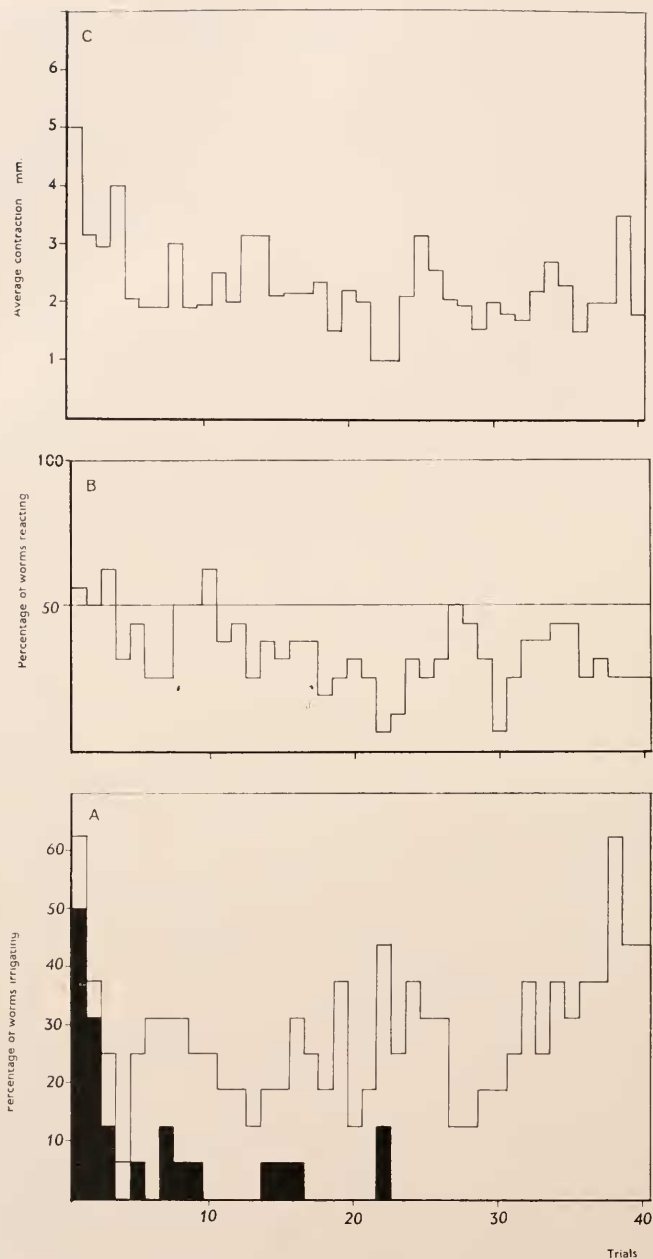


FIGURE 2. A, Percentage numbers of worms irrigating at each trial (empty blocks) and the percentage that stop irrigating when stimulated by sudden decreases in illumination (filled blocks); B, Percentage numbers of worms responding to a series of sudden increases in illumination; C, Mean contractions of worms responding to sudden increases in illumination.



individual performance that habituation is occurring at all (Fig. 3B) and, even in more typical performances, apparently habituated worms may suddenly react to the stimulus (Fig. 3C). Several factors may contribute to this irregularity: the influence of the length of the worms at the time of stimulation has already been discussed, and from observations it seems that worms extended from the ends of their tubes are particularly sensitive to stimulation, whereas individuals crawling along their tubes or reversing in them are relatively insensitive.

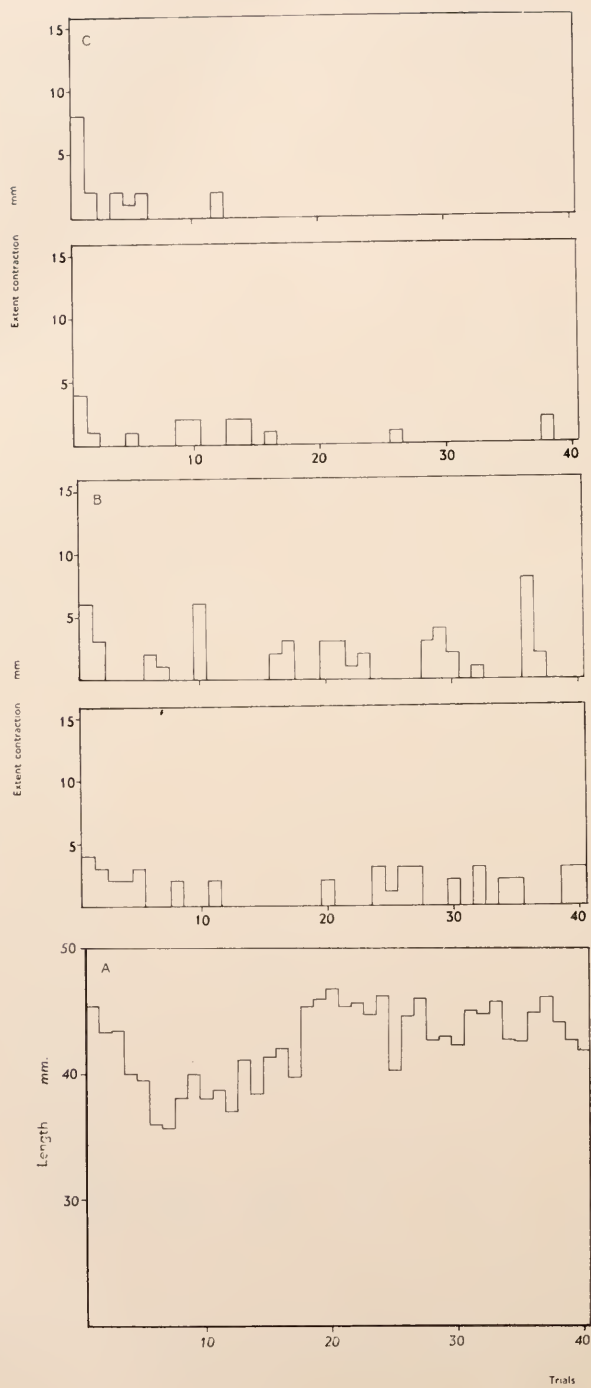
### DISCUSSION

Habituation in *Nereis* is probably a complex, central process which cannot be solely attributed to either sensory or motor accommodation. Accommodation of synapses may be an integral part of the process, and it has already been shown that sensory-giant axon synapses and motor-giant axon synapses accommodate to repeated electrical and tactile stimulation in *N. virens* (Horridge, 1950) and to sudden changes in illumination in *N. virens*, *N. diversicolor* and *Platynereis dumerilii* (Gwilliam, 1969). However, sensory adaptation is precluded from being entirely responsible for habituation, because the withdrawal response may be replaced during the habituation process by alternative behavior. Withdrawal to a posterior tactile stimulus is replaced by the posterior end bending away from the source of stimulation in *N. virens* (Horridge, 1950), and withdrawals to anterior tactile stimuli are often replaced by worms (*N. pelagica*, *N. diversicolor*, and *P. dumerilii*,) attacking the seeker used to stimulate them (Evans, 1969).

The withdrawal response itself changes in nature during habituation, and it is likely that one response is replaced by another before there is complete failure to respond. The initial extensive contractions are almost certainly giant axon responses, except possibly the responses to sudden increases in illumination which are slower, but the incomplete contractions that replace them are less likely to be mediated through the giant axons. Indeed, Gwilliam (1969) has made electrophysiological recordings from the ventral nerve cords of nereids and has found that giant axon potentials may occur to the first few stimulus presentations (light or mechanical shocks), but when these disappear bursts of fine fiber activity can still be recorded from the cord when the animal is stimulated.

Worms which have habituated to one stimulus are still capable of responding if the nature of the stimulus is changed (Clark, 1960a) so that neuro-muscular synaptic accommodation has also been excluded as an explanation of habituation. However, accommodation of this junction could act as a safety device if there was a continuous barrage of different stimuli which might otherwise lead to overstimulation of the withdrawal response.

There is evidence that habituation is a central, rather than peripheral, process in other invertebrates. Dethier (1952) found that habituation of proboscis eversal and drinking elicited by tarsal stimulation with a sucrose solution, in the blowfly *Phormia regina* could "spread" from one leg to the contralateral leg. A similar "spread" of habituation has been observed in the earthworm by Kuenzer (1958). Habituation to a tactile stimulus on one part of a segment extends to other parts of the segment and to neighboring segments in diminishing strength as the distance from the original stimulation increases.





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#### SUMMARY

1. Habituation of the withdrawal response in *Nereis diversicolor* has been investigated by photographing successive responses to sudden decreases and sudden increases in illumination.

2. Responses to sudden decreases in illumination are usually anterior end withdrawals whereas sudden increases usually elicit posterior end withdrawals. Withdrawals to sudden decreases are faster and have shorter latencies than those to sudden increases.

3. Most worms responded to the first stimulation in a series by withdrawing, but the number responding diminished with successive trials. Although there was an initial decline in the extent of withdrawals, subsequent contractions were approximately constant.

4. Sudden decreases in illumination sometimes inhibit irrigating movements, particularly when the stimulus is novel. However, habituation is rapid and apparently independent of habituation of the withdrawal response; after the third trial irrigating activity was infrequently inhibited by stimulation although withdrawals continued to occur.

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FIGURE 3. A, Mean lengths of worms, subjected to sudden increases in illumination, measured at the onset of each trial; B, Erratic responses of individual worms, upper figure: to sudden decreases in illumination, lower figure: to sudden increases in illumination; C, Typical habituation of individual worms, upper figure: to sudden decreases in illumination, lower figure: to sudden increases in illumination.

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